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STUDIES ON MICHIGAN CELERY DISEASES, II

Under Direction of G. H. Coons

A Study of the Early Blight Fungus, Cercospora apii Fres.

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FOREWORD

The importance of the celery crop in Michigan has led to extensive and intensive studies of the diseases to which this crop is subject. Bulletins have already been published bearing on the Late Blight and on the Phoma Root Rot. The present bulletin treats of the disease known as Early Blight. It presents a technical study of the causal organism and its physiological relation to the host plant, to temperature, reaction of medium, etc., as well as outlining methods of control.

The work embodied in this bulletin was carried out by L. J. Klotz working under the direction of Dr. G. H. Coons, as part of the work required for the Master's degree.

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L. J. KLOTZ

INTRODUCTION

The importance of celery as a truck crop in Michigan, Florida, New York, California, Colorado, New Jersey, and several other states is well recognized. The peat lands of Orange county, California were set to over three thousand acres of celery in 1910 (57). Throughout this country the words "Kalamazoo" and "Celery" have been closely associated for the past twenty-five or thirty years. Kalamazoo, Kent, Muskegon and Ottawa, are the counties in which the industry is most developed in this state. Considerable celery is also grown in truck gardens near Bay City, Detroit, Newberry, and in smaller quantities by truck growers through the state.

The possibility of the further development of the industry is indicated by the fact that there are in Michigan many more acres of muck and other lands available for celery growing. The obtaining of seed from many sources, domestic and foreign, in which the natural enemies of the plant have long been known, together with the long continued raising of celery year after year without rotation, have led to the establishment in this state of several serious fungous diseases to which celery is subject. Among these is the disease known as early blight, the importance of which in hot seasons makes desirable its further investigation.

NAME OF THE DISEASE

The disease has been called by several common names among which are "celery rust" (33), "celery blight" (1,33, 43), "celery leaf blight" (4, 63), "sun blight of celery" (14), "early blight of celery" (18, 19), "celery leaf spot" (24), and "celery spot mould" (10). The name "rust" has long been discarded due to the fact that at least two true rust fungi have been recorded as attacking celery (64). The word "spot" is not characteristically descriptive because of the spotting of celery leaves caused by several other fungi. The disease would best be popularly known as the "early blight of celery" because of its early appearance during the growth period of the plant; that is, during the hot months of July and August. It should never be designated as simply "blight" due to the easy confusion with another destructive celery disease known as "late blight" which appears during the later, cooler part of the growing season (11, 57, 8, 9).

HISTORY AND DISTRIBUTION

The first report of the disease in the United States was made by Galloway (28) in 1881 in describing the loss of 10,000 plants in one locality. In 1888, he reported further upon the disease and stated that "the disease occasions greater injury in sections where the summers are long, hot and dry" and that the disease is unknown where the soil remained cool and moist.

The first report of the disease for Michigan is by Ellis and Everhart (1885) in which plants collected in Michigan by W. J. Beal (21) are described.

Prillieux and Delacroix (1890) reported the disease as occurring on celery in the experimental garden of the Agronomy Institute at Joinville le Pont, France (55). They stated that the fungus had already been reported on various other Umbellifers, particularly parsley. Note was made here also that as the weather becomes cool the disease becomes less virulent.

The first spraying experiments were carried on by Halsted (1891) and he obtained almost complete control of the disease by the use of ammoniacal copper carbonate (33). McCarthy (1892) also had success in spraying experiments as well as in the use of shade. He used liver of sulphur spray or sulphur dust (47).

These results were at variance with the results of Galloway who stated that proper cultural methods more readily prevented the disease than any of the various fungicides used (29). This attitude was followed by Davis of the Michigan Station who published Galloway's notes and added the recommendation of flooding the ground to keep it always soaked (14).

Rolfs (1896-1898) also advised flooding and trench irrigation, but assigned as a reason that wetting the leaves supplied the moisture necessary for spore germination. He obtained practically complete control by spraying with liver of sulphur or freshly prepared bordeaux mixture (58, 59).

Duggar (1897) grew the fungus in pure culture on celery petioles and bean stems (18, 19). Hume (1899, 1900) by placing celery leaves in moist chambers, sowing the leaf surfaces with conidia and then after 48 hours stripping off portions of the epidermis and examining under the microscope, demonstrated the entrance of the parasite through the stomata (35). As a means of control, Hume advised the use of 4-4-50 bordeaux mixture applied at weekly intervals. Because of its cheapness he preferred the bordeaux to liver of sulphur and he found also that the bordeaux had slightly more fungicidal value than ammoniacal copper carbonate.

Various other American workers have made notes upon the occurrence, prevalence and control of the disease, but the observations in general do not add particularly to the above summary (6, 8, 9, 23, 25, 36, 37, 39, 40, 41, 42, 50, 63, 65, 72).

The brief technical note by Atkinson (2) should be mentioned since it presents a good figure of the fungus and corrects the error of the figure given by Scribner in which the spores are shown incorrectly attached.

In the foreign work little is added to the results obtained by American workers. Massee described the fungus as the Celery and Parsnip Leaf Blight (45, 46) and Eriksson (1913) stated that the *Cercospora apii* caused spotting of carrots, parsnips and other Umbellifers (22). The scattered references in the literature indicate that the celery early blight has practically a world-wide distribution, reports of its occurrence coming from England, Germany, Austria, France, Italy, Siberia, Canada, South America as well as many states of this country.

ECONOMIC IMPORTANCE

The records of the Plant Disease Survey of the Bureau of Plant Industry show a distribution of the disease in 32 states. The loss caused varied by states and from year to year and ran from light loss to as high as 75 per cent in some instances. In general the losses seemed to be greatest in the California (2-10 per cent) and in the Florida producing sections* but occasional attacks in the northern states, such as those recorded for New York in 1917 have brought about considerable damage, (5-10 per cent).

The reports of losses collated by the Plant Disease Survey, especially the ones from Florida, prove that the celery early blight is of considerable economic importance. It is evident that at times the fungus can cause serious injury to its host wherever celery is grown. Given an abundance of plant food and plenty of rain, celery makes a good vigorous growth in spite of much early-blight spotting. Under these conditions the principal injury is due to disfiguration of the leaves, which necessities excessive trimming in the preparation of the plant for market. The spotted leaves, moreover, dry out much more rapidly than sound ones, thus causing greater deterioration in shipment. This condition coupled with the general unsightliness brings about additional loss to the grower.

HOSTS

Although in the foregoing citations there are frequent references which indicate that the same specific fungus is by many investigators considered responsible for the production of similar diseases in other umbelliferous hosts, there are no records of the results of work by which cross-infection was demonstrated. Thus far, the writer has been able to secure infection by *Cercospora apii* Fres. on only two hosts, namely celery (*Apium graveolens L.*) and Celeriac (*Apium graveolens L.* var. *rapaceum* D C.). Repeated inoculations with pure cultures of this fungus and long continued exposure to very badly blighted celery of carrot, (*Daucus carota L.*), coriander (*Coriandrum sativum L.*), and fennel (*Foeniculum vulgare* Hill.) gave negative results. Likewise it has been impossible thus far to secure infection on celery using the fungus isolated from carrot. It thus appears that the *Cercospora apii*

*In 1905, Rolfs reported 20 per cent to 80 per cent injury in 1906. Winters noted some damage in 1908 and 1910. Stevens reported the disease as very bad in 1912 (10-50 per cent infection) and as prevalent in the period from 1913 to 1917. In 1917 the loss was 100 per cent locally. In 1918 Hessler noted whole fields brown with blight. In 1919, Link and Jagger observed the effect of a "deluge of early blight upon celery," stating that the plants as a result were "short, small, and of much reduced market value." (From records of the Plant Disease Survey).

Fres. of celery is a strain or variety distinct from those forms causing similar spotting on other related hosts.

SIGNS OF THE DISEASE

General

The common term "blight" at once brings to mind the picture of a plant, whose outer foliage at least has the appearance of being scorched, as if by the hot sun. In fact, this is the sight presented by celery plants under the worst conditions of early blight (Plate III). With the coming of drought, diseased plants appear to wilt and droop more readily than healthy ones. The older stems soon fall and, coming in contact with the moist earth, become rapidly filled with the fungous threads which spread throughout the tissue, in part saprophytically. These fallen leaves and stalks are almost completely made up of fungus mycelium and this material soon becomes so completely covered by the large numbers of fruiting structures as to have a dark, grayish, velvety appearance. It may be said here that these fallen stalks and leaflets are a very important source of further infection of the crop.

On the Leaves

The first macroscopic indication of the fungus within the leaf is revealed by the presence of from few to many small, slightly yellowish or pale-greenish spots a millimeter or more in diameter. These may be noted in from five to eight days after inoculation, when conditions best favor the fungus. These small spots enlarge and coalesce into a rather indefinite yellow spot, the size of which depends upon the number of initial entrances of germ tubes, the time elapsed since infection, the climatic conditions occurring during the growth of the fungus within the leaf tissue, and the condition of the host plant itself. Under the conditions described as best for the fungus, lesions have been found to attain the diameter of from one to two centimeters within ten days after inoculation. These spots are, in general, approximately elliptical in shape, and cause a wrinkling or slight folding of the immediately surrounding healthy tissue. (Plate I, II.)

The border of the lesion is frequently, but not always, slightly raised, particularly where the edge of the involvement meets the larger veins of the leaflets, and at times there is a yellowing of the tissue extending two millimeters or more beyond the border of the brown spot. More spots are found beginning at the margins than nearer the midribs. As the nutriment of the affected area is exhausted the color of the spot changes, progressively, to a pale brown, a slightly reddish brown, and ultimately to a dull slate or ashen gray as the fruiting structures appear. As indicated under general signs of the disease, the presence of high temperature and moisture and a cutting short of the food supply are instrumental in bringing about abundant fructification of the fungus. Conidiophores under these conditions are capable of producing a crop of spores in from six to ten hours. In one experiment, at 12:00 P. M., a portion of a fruiting surface was found to have conidiophores which bore no spores, but when examined at 6:00 a. m. the following day it was found to have produced an entire new crop of typical conidia.

This was observed microscopically by using the procedure described later under the heading "Dissemination."

On the Petioles

Mention has been made of the presence of the fungus on the fallen stalks. Occasionally the active, living petioles are attacked, elliptical spots having a water-soaked appearance being produced. The greatest diameter of the ellipse extends in the direction of the length of the petiole. The stems frequently break over at these points of infection leaving the foliage beyond to droop and die (Plate III).

On the Fruit

Repeated examination, with a hand lens and with the microscope, of the seeds kept in stock at this laboratory has failed to reveal the presence of the early blight organism or any of its associated effects. Examination was made microscopically of the sediment obtained by centrifuging water in which celery seeds were suspended. What may have been conidiophores of the parasite were seen on several occasions, but the parts bearing the characteristic scars were missing. Because of the probable restriction of the causal organism to celery it is reasonable to believe the parasite is seed-borne. Undoubtedly bits of blighted material containing viable fungus find their way, in the course of seed collection, into stocks of seeds and are thus distributed. Further search should be made using material from other sources. However, McKay and Pool found the conidiophore tufts and spores of *Cercospora beticola* abundantly present on sugar beet seed (51).

ETIOLOGY

Proof of Pathogenicity

Single spore cultures of the organism (Plat IV), were obtained by the use of the ordinary poured plate procedure, spores being obtained at times from diseased leaves and at other times from artificial cultures. The Petri dish was inverted and by means of the low power of the microscope single, isolated spores of the fungus were located and marked by a small ring of ink. These were transferred immediately to cornmeal agar slants and others allowed to grow for two to four days and then transferred. The cultures thus obtained were used in many subsequent successful infection experiments. A typical infection experiment is here described.

Celery trash that had overwintered near the Botanical Laboratory was, on April 15, 1920, shaken over two large healthy plants in the rain* chamber, some of the refuse being left in contract with the sound leaves. Seven days later (April 22) spots were noted on the leaves (Plate II). These gradually assumed the pale brownish color of typical Cercospora lesions and by April 28 had attained an ashen gray appearance. Characteristic spores were first noted on May 3. Using spores from the plants thus infected, plates were poured May 4, 1920 and the organism isolated on May 10, 1920. On June 12, 18 celery plants and 4 celeriac plants were inoculated by placing some of the pure cultures

*A large glass case equipped so that a fine misty spray of water could be thrown upon plants.

obtained from this isolation upon healthy leaves. Thirteen days later, June 25, all inoculated plants showed extensive infection which was probably due to the distribution of the spores to them by air currents created by opening and closing the doors of the rain chamber. There were a few random spots on the check plants. This experiment has been repeated several times with similar results; the checks, however, being better isolated, showed no infection by the early blight fungus. In one infection experiment the organism was re-isolated and the single spore culture thus obtained was used with success in two subsequent inoculations.

Morphology

Mycelium—The mycelium of the fungus as it occurs within the host tissue is composed of irregularly septate, fairly heavily walled threads (Plate V). These vary in width from 2 to 3.5 microns, attaining a diameter of 4 to 5.5 microns in the case of the threads that make up the stroma-like masses from which the fruiting structures arise. The young hyphae are small tubes, nearly hyaline, having relatively thin walls with septa only at long intervals and having a finely granular, homogeneous-appearing protoplasm. As they grow older they become gradually pale brown in color, gnarly, tortuous, and very irregular in shape, form septa at shorter intervals, build heavier walls, and their protoplasm becomes more coarsely granular with a few large (.5 to 1.0 microns), highly refractive, globular bodies (Fig. 4-5, Plate V). The mycelium formed in culture varies greatly according to the food constituents and character of the medium. Figure 5, Plate V shows its growth on melilotus stems and Figures 3 and 6 in cornmeal broth of pH 5 and 8, respectively.

Sclerotium-like Bodies—Dark brown, sclerotium-like masses of threads, having a diameter of 20 to 50 microns, are found in the substomatal spaces (Plate V, Fig. 6). From these through the stomatal pore are thrust the conidiophores which wedge apart and soon obliterate the guard cells.

Conidiophores—The conidiophores are fused together in the region where they emerge from the leaf, forming a distinct fascicle (Plate VI, VII). They are a decided brown in color, usually with 1 to 2 rather indistinct septa near the base but not infrequently having cross walls at 10 to 15 microns intervals throughout their length. They vary from 40 to 180 microns in length, the average being 40 to 60 microns; their width varies from 3.5 to 5.5 microns. At the end of a mature conidiophore is seen a distinct scar, circular in outline and with a small circular dot occupying the center which indicates the point of attachment of a spore previously borne (Plate VII, Figs. 2, 3, 4). Older conidiophores have along their surfaces from 2 to 6 of these scars, with a bend or geniculation at each scar (Plate VII, Figs. 8, 12.) The explanation for the presence of these geniculations is that a conidiophore having borne a spore, on being brought into moist surroundings sends out from just beneath the scar a new growth. From this new growth a new conidium is abscissed, at a distance of from 10 to 30 microns from the first scar. This sympodial branching may be repeated several times, leaving as many

distinct knees. As many as six geniculations have been seen (Plate VII, Fig. 8).

Spores—The spores or conidia which are borne at the apices of the conidiophores are much elongated and slender (Plate VIII). They are spoken of as inversely clavate or obclavate in form; that is, the part next the point of attachment is greater in diameter than the free ends. Several writers have made the error of considering the spores as attached by their small ends (37, 63). A slight tendency toward chain formation was also noted (Plate VIII, Fig. 11j, k). Over 200 spores from several sources were measured, the dimensions being as follows:

Table 1—Spore Sizes of Cercospora apii

	Minimum	Maximum	Average
Length (microns)	22.0	290.0	55.–100
Width at base (microns)	3.5	4.5	4.0–4.5
Number of cells	2	30	4.—12

The conidia attained their maximum length in the warm moist atmosphere of the rain chamber where the humidity was high and prolonged. The spore contents are non-vacuolate, finely granular and subhyaline, the ones formed in pure culture being slightly more transparent than ones collected in the field. They are commonly constricted at a segment of the spore, this portion appearing devoid of protoplasm (Plate VIII, Fig. 11b). On culture media a few typical obclavate spores are formed and abjointed at first, but later the new conidiophore growth becomes longer and remains attached as hypha-like branch. These when mounted appear as long, slender filaments which may be considered atypical or abortive spores, (Plate VIII, Fig. 11h).

Name of Causal Organism

The causal organism, was first described by Fresenius in 1863 under the name *Cercospora apii* (26). Through error the credit for this was assigned by Scribner (63) and some others to Fries. To avoid mistaking the authority for the name the abbreviation should be written *Fres.* or *Fresen.* not *Fr.,* thus—*Cercospora apii* Fres. (of Fresen).

Life History of Causal Organism

The writer has twice proved that the fungus will overwinter on celery refuse. Microscopical examination of this refuse reveals the presence of the typical conidiophore fascicles, and leaves containing the overwintered parasite when placed in a moist chamber in the warm laboratory are soon covered by a new growth of typical Cercospora spores. Early in the spring of 1921 spores which had overwintered on trash were found. They were so very hyaline as to be difficult to see, were much swollen—5.5 to 6.5 microns at the base—and failed to germinate. This tends to discredit Massee's statement (45, 46), that spores themselves live through the winter and are capable of infecting a crop the following season. The fact that the fungus makes a good growth on sterile muck (Plate IIb) may indicate another means of overwintering and a source of infec-

tion the following season. (Growth on muck is described under "Cultural Characteristics.") The spore, under the suitable climatic conditions described, on coming in contact with a celery leaf germinates, sending its delicate, thread-like germ tubes into the leaf tissue. (See under "Infection Phenomena.") Here it grows, absorbing nutriment from the leaf cells and producing a well-defined spot. The nutriment at the center of the lesion being first exhausted, the fungus sends out fruiting hyphae which produce spores capable of immediately infecting the crop. This course continues throughout the hot, humid summer months until the coming of the cooler, rainy fall weather. Then the fungus within the fallen leaves and petioles assumes a resting stage in the form of the stroma-like masses of heavy-walled mycelial threads described. It thus continues its existence until the coming of hot, humid weather again.

RELATION OF PARASITE TO HOST

Mode of Infection

To demonstrate the mode of entrance of the organism, mature celery leaves were placed in a moist chamber and inoculated with spores and mycelium from diseased material. The points of inoculation were marked by means of small pieces of moist filter paper, each having a 5 mm. circular hole in its center. From time to time bits of the leaf epidermis were stripped off and examined. A mount made after 25 hours showed two distinct germ tubes from a piece of mycelium entering a stoma (Plate V, Fig. 1). Spore germ tubes were also seen entering the leaf tissue in the same manner, but the view was too indistinct to be traced by means of the camera lucida. The spore shown in Plate V, Fig. 1, was not seen in the mount illustrated but was placed there for the purpose of size comparison. Hume (1900) demonstrated stomatal entrance in a similar manner (35). The writer has as yet, not been fortunate enough to secure pieces of epidermis from young heart leaves which show this entrance; nor has he observed entrance through wounds. Although it is reasonable to believe that the fungus may enter through wounds, experiments show no increase in percentage of infection where the leaves have been wounded. That the parasite can gain entrance to the interior tissue of the leaf when placed on either the upper or lower leaf surfaces was demonstrated in infection experiments. This is to be expected, the stomata being present in both upper and lower epidermis.

By means of the method employed by Makemson (1918) (44), germ tubes were observed to enter leaves that were not detached from the plant. In this method the leaf is bent over onto a slide on the microscope stage and held in place by means of rubber bands. To supply moisture to the leaf surface a narrow ribbon of filter paper is employed. The end of this paper, which is placed over the leaf, has a small circular opening which marks the place of inoculation. The other end dips into a beaker of water set near the stem of the potted plant. Spores of the fungus are placed in the small exposed leaf area and over this is placed a cover slip. With low power, direct microscope observation is possible. Finding that insufficient moisture was supplied by the filter

paper, capillary glass siphons were used which supplied water to a small piece of filter paper strip kept on the leaf, at any rate desired, depending upon the diameter of the tube and height of the source of the water. In some experiments, for the purpose of viewing better the spores and germ tubes, the cover slip was removed after sufficient time had elapsed for entrance, and the fungus stained a minute with a 1 per cent aqueous solution of eosin (20), (31). The stain was removed from the leaf surface with filter paper and water, the paper strip removed, the cover slip replaced over the area of inoculation, and the fungus directly observed with both high and low power.

It was noted that the fungus most readily enters the leaf when conditions of high humidity are present in the surrounding air. However, where the water forms a visible film over the leaf surface, the germ tubes do not penetrate the stomata. It was noted with spores germinating upon a glass surface that where a moisture film enveloped the germ tube, this film was pulled along with the extending tube. This small film enveloping the spores may be significant in the infection process. It is likely that the tube extends the enveloping water film until this film is brought in contact with a stomatal opening into which the water is quickly drawn by capillary action. Thus the germ tubes would build continuous water passageways from the spore to the inner parenchyma of the leaf. The factors involved in the establishment of the parasite within the host after invasion have not been determined. Entrance may be effected in from six hours upwards, depending upon the rapidity of germination and the proximity of the stomata.

Although entrance of the fungus into young heart leaves has not been seen, it is undoubtedly true that this does take place. Pool and McKay attempt to correlate age of the leaf and stomatal activity with the susceptibility of beet leaves of different maturity to the leaf spot fungus, *Cercospora beticola* Sacc. (52, 53). The stomata of immature celery leaves being less frequently open and having smaller slits than those of mature leaves are facts which must be taken into account when we consider that heart leaves infrequently show eivdences of blight. To note accurately the relative susceptibility of young and old leaves, the heart leaves of four healthy plants were tagged March 31, 1921; the plants were placed in the rain chamber and inoculated with spores from blighted leaves. Ten days later pale-yellowish spots 1-3 mm. in diameter appeared at the margins of some of the heart leaves. As the spots did not appear to enlarge, some of the heart leaves bearing them were placed in moist chamber April 16. April 19 the lesions had enlarged to areas measuring about 5 mm. by 8 mm. Examination April 21 revealed them bearing typical conidiophores and spores. By April 23 the diseased heart leaves remaining in the rain chamber had borne spores. From this it appears that the young immature leaves of celery are capable of being entered by the organism which, however, is unable to cause a definite, easily recognizable Cercospora lesion within twelve to sixteen days. It seems that after the parasite has gained entrance it makes but little apparent progress while the leaf is actively growing. It is evident from many observations that the fungus makes its most rapid progress in the old retrograding leaves. On mature leaves spots are produced in from five to eight days, whereas it takes at least 10 to 14 days on the immature heart leaves.

On April 15 the heart leaves of four plants were inoculated with diseased material and tagged for later identification. Eighteen days later these leaves had attained almost their final mature size and bore the characteristic fruiting bodies of the fungus.

Relation to Light

To determine whether darkness has any influence on the infective powers of the fungus two potted plants were placed in a dark constant temperature apartment and inoculated with diseased material. One plant was kept moist by inverting over it a battery jar, the lower half and bottom of which were lined with moist filter paper. Some leaves of the other plant were kept moist at the points of inoculation by means of wet absorbent cotton wisps, while others were irrigated with capillary siphons after the manner described in the study of the mode of infection (pp. 10 and 11). The uninoculated leaves were considered as checks. Plants were similarly treated in the light of the laboratory. Six days later some of the inoculated leaves began to show signs of infection. Spotted leaves were placed in moist chamber and four days later were observed to have produced typical conidiophores and spores. These results are in contrast to the observations by Pool and McKay (52, 53), who worked with the Cercospora leaf spot of sugar beet. They reported that infection probably takes place only in the day time and that immature leaves are not susceptible.

Water and Temperature Relations

Once established within the host leaves the fungus makes rapid progress during hot, dry weather. The factors which most favor the fungus, as determined by field observations and by controlled conditions in the greenhouse, are high temperatures (80° to 90° F.) coupled with a droughty condition of the soil.

When we consider dissemination of the causal organism, we must take into account two other factors; air currents of sufficient strength to distribute the fungous conidia, and enough moisture on the celery leaves to permit of the germination of the spores when they arrive. Dews, a high humidity, and light rains supply this moisture requirement of the fungus. One notes a most rapid spread of the disease and fruiting of the fungus in hot, muggy times following a period of drought. To demonstrate the germination of spores in condensation water the method of Duggar (page 59, 19) was employed. A van Tieghem ring was fastened to a slide with vaseline and paraffin, a few drops of water placed in the ring, and over this inverted a cover slip on the top of which had been placed a number of conidia. The spores readily germinated in the moisture which condensed on the glass, germ tubes appearing in from 2 to 5 hours at a room temperature of 28° C. (Plate VIII, Figs. 3, 4, 5.)

In the greenhouse, proper conditions for a thorough infection of healthy plants were secured in the rain chamber which is a plateglass cage (Wardian case) approximately cubical, having a capacity of 12 cubic feet. Entering this through a hole in one corner of the bottom and extending upward until it nears the center of the glass top is a half-inch water pipe, terminating in a small opening from which a small jet of water emerges forcibly. This water is directed against the

top of the small chamber where, breaking into a fine spray, it thoroughly wets the leaves of plants placed therein but does not supply sufficient water to the soil to injure the plants. Healthy potted plants were placed in this apparatus, the mist turned on for a half a minute, and the plant "peppered" with spores by shaking diseased material over them. The leaves were kept moist for a period of 8 to 10 hours by turning on the mist for a few seconds at intervals of about two hours. The chamber was maintained at a high temperature by means of two 16 candle-power carbon-filament lights. Making use of this apparatus, conditions were obtained which best simulate those in nature when early blight is most prevalent and destructive. Knowing these conditions we are thus better able to understand and explain the severe losses experienced in Florida and other localities.

Pathological Histology

The character of the mycelium as it occurs within the leaf was described under "Morphology" Mention was not made as to the means by which the parasite obtains its nourishment. The mycelium viewed in mounts of small portions of diseased leaves can, by careful focussing, be seen to possess small knob-like structures 2 to 3 microns in width (Plate V, Fig. 4). These outgrowths or haustoria of the fungus mycelium extend through the cell wall and lie in close contact with the cell contents, absorbing nutriment therefrom for the organism. The hyphae as seen in stained paraffin sections of blighted leaves and stems are exclusively intercellular, although in the very old, disintegrated tissue they appear to pass right through the cells ramifying, seemingly unobstructed, as in the growth on artificial culture medium. An abundance of hyphae shows evidence of a rapid growth throughout the sponge parenchyma and palisade tissue, here readily wedging the cells apart. Distortion of the host tissue is most evident in the regions where the coarse, heavy-walled threads collect to form spherical, stroma-like masses. Guard cells of the stomata are forced apart as the conidophore bundles are protruded, and are overshadowed by the short, immature stalks of the bundle, and soon lose their identity. Plate V, Fig. 2 illustrates the beginning of this procedure, and Plate VI and Fig. 1 of Plate VII the ultimate effect. The bases of the fascicles were found to measure as wide as 50 microns whereas the slits of mature stomata are only 10 to 13 microns in length.

Cultural Characteristics

In pure culture the fungus was found to grow well on all media employed. Plantings from cornmeal agar cultures were made as shown in the following record, and the growth observed from day to day. The effects of light and darkness on growth and sporulation were also studied at the same time. For growth in darkness, a ventilated metal chamber was used. The diffused light of the laboratory supplied a second lighting effect, and a 50-watt Mazda bulb in the dark room of the laboratory gave a third condition of illumination. In the second and third cases the cultures were placed in a single row to give the full effect of the light to each tube. The character of the growth was observed frequently but the results here given are those recorded at the end of the experiment. Not considering the amount of growth, the microscopic appearance of the

fungus varied but little from time to time. The color of the small mycelial threads is a translucent gray. As they become older the hyphae manufacture a pigment which varies in color with the character of the medium. On some media it is a dark gray to black, on others shades of brown, but it is generally a deep chromium or mineral green. The dark-green pigment, so pronounced on cornmeal agar, was found by the microscopic examination of sections to be entirely within the mycelial threads and not in the medium. The mycelium penetrates the agar to a depth of .5 to 1.5 cm., the color not being present in the young branches which make the borders of the growth (Plate IV).

Light Relations

The following record was made after ten days' growth on the various media. The growth in darkness and diffused light took place between 20° C. and 24° C.; that in electric light was necessarily a little higher (23°-26° C.).

Table 2—Growth of Cercospora apii on Various Media

1. Turnip plug: Darkness—The superficial growth covered about one-third the surface of medium and was a dull mouse-gray in color. Prominent folds and bunches of a deep-green pigmented growth in the medium. This extended to a depth of .5 cm. No typical spores found.
   Diffused light—As in darkness. No typical spores.
   Electric light—As in darkness. No typical spores found.
2. Rice: Darkness—Mouse gray as in (1). In medium a chromium-green pigment 2 mm. deep. No. typical spores found.
   Diffused light—Similar to that in darkness but aerial growth slightly lighter colored. Typical spores not found.
   Electric light—As above. A few typical spores.
3. Potato plug. Darkness—Aerial growth not distinct. Considerable dull, dirty, yew-green growth covering one-fourth of surface of plug. Less elevated than (1) and (2). Spores none.
   Diffused light—Slightly more elevated and folded.
   Electric light—As in diffused light.
4. Bean pods: Darkness—Considerable dull, intensely black growth with short velvety mouse-grayish aerial growth on the many folds and bunches. No typical spores.
   Diffused light—Exactly similar to that in dark.
   Electric light—Exactly similar.
5. Corn Meal: Darkness—Considerable, the greater portion being submerged and having a dark chromium-green pigment which is most intense near center of growth.
   Diffused light—As in darkness. A few typical spores found.
   Electric light—As in darkness.
6. Oat Meal Agar: Darkness—An abundance of dull-black growth, citron green at the border. A few clumps of gray aerial mycelium. No typical spores found.
   Diffused light—Similar.
   Electric light—Similar.
7. Nutrient Broth: Darkness—Characteristic growth of (5) at the surface of the liquid and on the glass. Many small rainette-green submerged colonies against the glass. No spores.
   Diffused light—Same.
   Electric light—Same.
8. Carrot plug: Darkness—Tomentose, mouse-gray aerial growth and a tough folded black growth in substrata. Spores abundant.
   Diffused light—More abundant growth and spores.
   Electric light—As in diffused light.
9. Nutrient Dextrose agar: Darkness—Black in substrata, mouse-gray to a faint pinkish-gray to almost white aerial growth. Exuding amber colored drops of liquid over white aerial mycelium spores.
   Diffused light—As in darkness, but with more numerous amber and colorless moisture droplets. Spores two plus.
   Electric light—Similar.
10. Potato broth: Growth very similar to that in Nutrient broth. Submerged growths more fluffy.
11. Celery Agar: Darkness—Moderate amount (one-half of surface). Mouse-gray to white aerial mycelium in clumps, simulating conidiophore fascicles. A black-green pigmentation. No spores.
    Diffused light—Same as in dark.
    Electric light—Same as in dark.
12. Prune Juice Agar: Darkness—Abundant mineral-green to black growth with small mouse-brown cottony clumps, of aerial mycelium. No spores found.
    Diffused light—Same. Aerial mycelium green near the point of planting.
    Electric light—Same as in diffused light.

13. Corn Meal Agar: Darkness—As in (11) but with fewer, more scattering conidiophore-like clumps. Spores two plus.
Diffused light—Same.
Electric light—Same.

14. Nutrient Agar: Darkness—Abundant, deeply submerged, (.5 cm.) black growth with mouse-gray to white aerial clumps. No spores found.
Diffused light—Same but with less white aerial mycelium.
Electric light—Same as in diffused light.

15. Nutrient Sucrose Agar: Darkness—As in nutrient glucose agar, with amber droplets exuding from white aerial mycelium. Spores two plus.
Diffused light—Similar, but no moisture droplets. Spores two plus.
Electric light—Aerial growth shows a slightly pinkish coloration. Spores two plus.

It is evident from the above record that light effects are not very marked. Corn meal agar cultures in preparation dishes were later similarly exposed to light and darkness with similar results.

To repeat the above observations on the effect of the absence of light on the growth of the fungus in pure culture, five corn meal agar slants were planted with mycelium and placed in a black wooden chalk box. This was enclosed in a pasteboard box and the whole set near a north window of the laboratory. Three similar plantings were placed in a wire basket and set alongside of the boxed tubes. Two weeks later the cultures were examined. There was no apparent difference in the growth of the fungus except that the aerial mycelium of the tubes kept in the darkness was a slightly darker gray. Typical spores were produced in both groups of tubes.

Growth on Muck and Celery Refuse

The record given above shows that several media which supply the food necessary for a good growth of the fungus in pure culture may be employed. Particularly may be mentioned carrots, the nutrient sugar agars, rice, and corn meal agar. Cultures on carrot and rice are rather difficult to transfer rapidly, due to the tough, resistant growth. The sugar agars, while being convenient to prepare, are good media for bacterial as well as fungous life. They could not be used to good advantage in isolation work. Because of its convenience in handling and because of its apparent suitability as a food for the isolation of the organism, corn meal agar was the medium most employed in this work (Plate IV). Good growth of the fungus was secured also on sterile lettuce, celery refuse, beets, orange and lemon peelings, Coons' cheap synthetic medium,* Richard's synthetic, and muck.

To determine whether or not the organism would grow on sterilized muck and celery refuse, and to note the character of the possible growth as influenced by three different treatments of these natural media, the following was performed:

Five separate portions of quantities of muck, muck and refuse in about equal volumes, and refuse alone were treated respectively as follows: One set of the materials was placed in preparation dishes and distilled water added until it was within .5 cm. of the surface of the medium. This set, was called "saturated." Another group was treated with just sufficient water to give the soil a nice friable condition, the material put in preparation dishes and labeled "Optimum water content." A third series was treated as the second, using filtered lime water, and marked "Lime water." A fourth was saturated with water containing surfuric acid in the proportion by volume of 250 of H_2O to 1 of

*Mich. State Bd. of Agr. Rept. 1919: 260.

H_2SO_4; the preparation dishes containing the media thus treated were called "acid." The fifth set, used as a check, was left untreated. All were autoclaved at 20 pounds pressure for forty-five minutes and planted with mycelium from a corn meal agar culture. The results are shown in Table 3.

This experiment simply shows further the saprophytic nature of the organism and suggests the possibility of the fungus over-wintering, if not persisting longer, in the soil and being capable of infecting a celery crop the following summer (Plate IIb).

Temperature Relations

The season in which the disease appears in the field, together with its reactions to controlled conditions in the greenhouse suggest a rather sharp sensitiveness to heat. To obtain further knowledge of the temperature relations of the fungus, use was made of a model of a Ganong differential thermostat. This consists of two galvanized iron chambers, one for a freezing mixture and the other for boiling water, which are connected by a square trough of the same material. This trough is divided into nine small compartments by means of cardboard partitions and is covered by two pieces of glass, making a gable-like effect. Each division is equipped with a small immersion thermometer. Ice was renewed each day in the one chamber, and the water in the other, kept boiling by means of an electric immersion heater, was automatically supplied from an aspirator bottle.

Table 3—Growth of Cercospora apii on Muck and Celery Refuse

Medium and Treatment	Growth	Spores
Optimum Water Content		
1. Muck	Medium ¾ covered with mouse-gray and white growth, the white immediately around the point of inoculation and amounting to ⅓ the viable fungus.	None found
2. Refuse	Entire medium covered by a brownish-gray plushy growth.	Many
3. Muck and Refuse	Surface ¾ covered. Character of growth as in (1) except less white area.	Found on refuse
Water Saturated		
4. Muck	Surface ¼ covered. Character as in (1).	
5. Refuse	As (2) where medium is out of water	Abundant, some germinating.
6. Muck and Refuse	Nearly entire surface covered as in (1)	Few spores found on refuse.
Lime Water		
7. Muck	As in (1)	Few atypical spores found.
8. Refuse	As in (2)	As in (5)
9. Muck and Refuse	As in (3) with a little more white growth	As in (3) but fewer
Acidified		
10. Muck	Character of growth as in (1), ⅓ surface covered	Few
11. Refuse	½ surface covered as in (2)	Many
12. Much and Refuse	As in (9), ½ of surface covered	Few
Untreated		
13. Muck	Appearance as in (1), ½ covered	Few
14. Refuse	As in (2)	Many
15. Muck and Refuse	Character of (3), more growth being on refuse than soil	Found on refuse

Vegetative Growth—Making use of the apparatus described above, the series of temperatures recorded in the following tabulation was obtained. Plantings were made on corn meal agar in preparation dishes and four cultures placed in each compartment. The temperatures of the several compartments were recorded and the growth of the cultures observed each day for 16 days. The dishes were then removed and measurements and observations recorded.

Table 4—Temperature and Growth of Cercospora apii

No. of Compartment	Temp. Range of Compartment (°C.)	Diameter of Growth, mm.		Growth	Spores
		Max.	Min.		
1	41–43	....	....	Death	
2	36–38	7.5	4.0	Poorest. Heavy, resistant mycelial threads	–
3	30–31	28.0	25.0	Next best	+Few
4	27–29	34.0	31	Optimum	+
5	24–25	23	23	Good	+ Few
6	22–23	27	22	Good	+Few short spores
7	20–21	18	18	Declining	++Many more
8	15–17	17	11	Not so good as 7	+++ Abundant
9	4–8	6	5.5	Poor	+ Few

These data indicate that the fungus grows well between 23 and 30° C., temperatures above or below this range being distinctly opposed to the best progress of the organism. Most abundant sporulation took place between 15 and 21° C. In several attempted repetitions of this experiment upon carrot disks, Coons' synthetic medium* and corn meal agar, the cultures were contaminated by mites in spite of a thorough dusting of the apparatus with Pyrethrum powder and spraying with nicotine solution. Observations of test tube cultures confirm the above results except that the greatest numbers of spores were found at 20-21° C. Work with the living host was impossible because potted celery plants small enough for the compartments did not survive there long.

Spore Germination—Spores and conidiophores were removed from diseased celery, placed in tap water on a cover slip and inverted over van Tieghem rings as already described (19). They were then exposed for 14 hours to the temperatures shown in the table and observations made and recorded.

*Journ. Agr. Res. 5: 752.

Table 5—Spore Germination of Cercospora apii at Various Temperatures

Temp. (°C.)	New Conidiophore Growth	Spore Germination
42–43	.....	Death
35–36	About half the number germinated. Growth 16–100mu in length; one was 120mu	60% germinated. Tubes 30–85mu long
27–29	Many (50%) germinated; 170–190mu long	100%. Practically every cell showed a germ tube
22–24	(50%) 60–120mu long	As at 27–29°, but fewer cells germinated
19	(50%) Growth 30–40mu long	100% tubes 100–170mu long, fewer cells germinated than at 22–24°
16	Few (10%) tubes 16–50mu	Many (60%) tubes 38–70mu. Some attaining 85mu in length
11	.....	Small germ tubes on many (60%) 14–20mu
8–10	.....	.....

This indicates that new growth is rapidly made in temperatures ranging from 22 to 29° C. At 27° to 29° the greatest quantity of growth was put out and at this temperature, moisture conditions being optimum, conidia are no doubt most efficient in infecting the host.

Thermal Death Point—The thermal death point of mycelium as grown in corn meal agar was determined by means of Novy's capillary tube method (51). By crushing the agar of a culture with a sterile scalpel the mycelium was broken into relatively small bits. Five cc. of sterile distilled water were then added and the test-tube vigorously shaken making a suspension of the broken hyphae. Sterile capillary tubes, 4 inches in length, were dipped into this suspension which was permitted to rise 4-5 centimeters. The capillary tube thus charged was sealed at both ends in the flame of a microburner. Using heavy iron water baths, the temperatures of which were carefully regulated by the size of the flame below and by having the room as nearly devoid of air currents as possible, the capillary tubes were exposed as shown and then discharged onto corn meal agar in petri dishes.

Table 6—Thermal Death Point of Cercospora apii Mycelium

Temp. °C.	Time of Exposure				
	1 Min.	2 Min.	5 Min.	10 Min.	20 Min.
40	+	+	+	+	+
45	+	+	+	+	+
50	+	+	+	—	—
55	—	Contaminated	—	—	—
60	—	Contaminated	—	Contaminated	—
65	—	—	—	—	—
70	—	—	—	—	—

+ = Fungus growth in the cultures at close of experiment.
— = no growth in the cultures.

Using aerial mycelium and spores, scraped from corn meal agar slant cultures, the above was repeated. In this instance the temperature intervals were smaller and the capillaries exposed for 10 minutes in all cases.

Table 7—Thermal Death Point of Cercospora apii Mycelium and Spores

Capillary No.	Temperatures							
	42	43.5	45	48	50	51	52	54
1	+	+	+	+	+	+	—	—
2	+	+	+	+	+	—	—	—
3	+	+	+	+	—	—	—	—

For an exposure of ten minutes the thermal death point of the fungus mycelium, therefore lies between 50 and 52° C. Two of the three ten-minute exposures at 51° C. were lethal.

As it was found impossible to obtain a sufficient number of typical spores and conidiophores from pure culture to carry out the above work satisfactorily, these fruiting bodies were obtained from diseased leaves. They were removed with small forceps and placed in 2 cc. of water in a preparation dish cover. The suspension thus made was taken up with capillary tubes subjected to temperatures as above described and discharged on cover slips and inverted over van Tieghem rings. The hanging drops were examined at intervals for three successive days and germination or its absence noted.

Table 8—Thermal Death Point of Conidiophores and Conidia of Cercospora apii

Conidiophores, Tube No.	Temperatures, °C.							
	42	43.5	45	48	50	51	52	54
1	+	+	+	+	+	—	—	—
2	+	+	+	+	+	—	—	—
Conidia, Tube No.								
1	+	+	+	+	—	—	—	—
2	+	+	+	—	—	—	—	—
3				—	—	—	—	—
4				—	—	—	—	—

Spores of two of the four capillaries exposed to 48° C. for 10 minutes failed to germinate; conidiophores put out no new growth after the 51° C. exposure. While the experiment is faulty, in that a pure culture was not employed, it at least indicates something of the relative reaction to high temperatures of spores and the heavier walled mycelium.

Relation to Reaction of Medium

Germination or non-germination of spores in several solutions which have a range of hydrogen-ion concentration values give an indication of the relation of the parasite to the reaction of the medium.

As a preliminary test conidia were placed upon corn meal agar, in distilled water, and in city water, and the rapidity of germination noted by measuring the length of the germ tubes from time to time. The results are recorded on Plate IX. In the same period of time the germ tube of the spores on corn meal agar attained greater growth than those in tap water, those in tap water in turn growing faster than the ones in distilled water. The pH of the agar was 6.7, that of the tap water 7.6, while the distilled water was pH 5.7. This small preliminary

test may give a slight indication of the effect of hydrogen-ion concentration and possibly of the food and character of the medium on germination.

Corn meal broth solutions were next adjusted to different hydrogen-ion concentration using hydrochloric acid and sodium hydroxide solutions, spores mounted therein and germination observed. The range as determined by Gillespie's* colorimetric method was pH 4 to pH 8. Solutions having a higher H ion concentration than pH 5 inhibited germination.

To eliminate, more or less, the food and other factors, spores were placed in various buffer solutions of known hydrogen-ion concentration and observed from time to time. The buffers used, which were made according to the formula of Clark,** are the following: Their accuracy was tested electrometrically.

1. Primary potassium phosphate—Sodium hydroxide solution. Spores germinated in the entire range of pH values given by this solution (pH 5.8-8.0), the germ tubes attaining greatest length at pH 7.

2. Acetic acid—sodium acetate mixture—pH 3.6-5.6. No germination took place at any value. It was reasoned that the concentration of the hydrion was so high as to be detrimental to germination, or that the salts themselves were toxic, or that both factors combined were instrumental in preventing growth. That the high concentration of the hydrion alone was not responsible in some mounts is shown by the third buffer used.

3. Potassium acid phthalate—sodium hydroxide (pH 4.-5.6). Conidia did not germinate below pH 4.6 but did from pH 4.6-5.4.

The conclusions to be drawn are evident. Many more buffer combinations must be tried to reduce the paradoxes due to the toxicity of the solution itself to a minimum. Quantities of corn meal broth or some other medium that proves satisfactory should be treated with various amounts of appropriate acid and alkali or suitable buffer, and the pH values determined from time to time by the accurate electrometric method. Having thus obtained solutions of media having a long range of hydrion concentration values, the organism should be placed in them and observed as to germination and growth. There are probably so many and complex factors to be taken into account to attain these hypothetical media as to make them impossibilities. Trust could not be placed in such an unknown quantity as corn meal extract. Its reaction varies on solution with the progressive hydrolysis of its various components. Nevertheless, a procedure very roughly simulating the above was carried out.

To definite portions of sterile corn meal broth (pH 6.7) were added varying quantities of autoclaved hydrochloric acid of known strength, and the hydrogen-ion concentration determined colorimetrically, making use of Medalia's† short-cut system. This was repeated, adding aseptically to proportional quantities of the broth, corresponding amounts of the acid, giving stock quantities of the medium whose reactions were

*Gillespie, L. J. The reaction of soil and measurements of hydrogen-ion concentration. Jour. Wash. Acad. Sci. 6 : 7-16. Figs. 2. 1916.
Gillespie, L. J., and Hurst, L. A., Hydrogen-ion concentration measurements of soil and two types: Carbou loam and Washburn loam. Soil Sci. 4 : 313-319. 1917.

**Clark, W. M. The determination of hydrogen-ion, 1-317, Figs. 1-38. 1920.

†Medalia, L. S. Color Standards for the Colorimetric Measurement of H-ion Concentration pH 1.2 to pH 9.8. Jour. Bacter 5 : 441-468. Figs. 1-4. 1920.

found to be from pH 6.7 to 1.9. To obtain the alkaline range the process was repeated using sterilized N/4 NaOH. Five cc. portions of the stock solutions were added aseptically to sterile test tubes each containing a small filter paper cone. Two transfers were then made for each pH value, planting a small piece of fungous mycelium near the apex of each cone. The inoculations were made February 1, 1921, and growth was observed from time to time as shown in the following table.

Table 9—Growth of Cercospora apii in Media of Various Reactions

pH Values	Growth (1 week) 2-8-21		Growth 2-14-21		Growth 2-24-21		Spores
1.9	—	—	—	—	—	—	
2.1	—	—	—	—	—	—	
2.3	—	—	—	—	—	—	
2.5	—	—	—	—	—	—	
3.0	—	—	—	—	+ Very slight	+	No normal spores found. A few atypical
3.5	—	—	—	—	+ Slight	+	
4.0	—	—	+ Slight	+	+ .6 cm.	+	
4.5	+ .7 cm.	+ .7 cm.	+ 1 cm.	+	+	+	
5.0	+ 1 cm.	+	Sclerotium-like mycelial mats + 1 cm.	+	+	+	
5.5	+	+	+	+	+	+	
6.0	+	+	+	+	+	+	
6.38	+	+	+ 1.3 cm.	+	+	+	
6.7	+ 1.2 cm.	+	+	+	+	+	
6.9	.....		Contaminated		.....		Spores
7.2	+ 1.2 cm.	+	+ 1.5 cm.	+	+	+	
7.6	+	+	+	+	+	+	
8.0	+ 1 cm.	+	Sclerotium-like mycelial mats +	+	+	+	Largest number typical spores
8.4	+	+	+	+	+	+	
9.0	+	+	+	+	+	+	

The inability of the fungus to make noticeable growth at once at pH 4, 3.5 and pH 3 and then later to make appreciable growth at these concentrations of the hydrion seems to be due to some change in the medium. This was due either to the effect of the fungus or the instability of the medium itself, or both. However, titration of the stock quantities at the end of the experiment showed that the hydrogen-ion content of the medium had varied greatly. That which tested pH 1.9 at the initial determination showed pH 4 at the final, pH 8.4 media had fallen to pH

7.6, 2.5 had gained 2 and so on. Only those solutions near neutrality showed any constancy. We may say that the figures only in a general way indicate a tolerance of the organism toward the acidity and alkalinity of the medium in which it was grown. It is obvious that exact descriptive statements and conclusions are here impossible, due primarily to the varying of the very factor under consideration.

To observe something as to the ability of the fungus to change the reaction of the medium there were added to 5 cc. portions of corn meal broth (pH 6.7) the necessary quantities of the various indicators and the organism planted therein. One week later the colors showed the hydrion concentration of the broth to have increased to pH 6.5, and two weeks from the date of the last record showed pH 6.2, and five weeks after planting 6.5. The last record, made 10 weeks after the initial was pH 6.6.

Hydrogen-ion concentration values for the juice expressed from celery leaves and carrot leaves and for water extracts of the celery powder used in the chemical determinations were determined electrometrically. The writer is indebted to Dr. C. S. Robinson of the Chemical Section of the Experiment Station for these data. These represent values for material from only two sources and are not in any way to be considered comprehensive. The leaves were removed from the plants, washed in distilled water, and then subjected to 250 pounds pressure per square inch in a hydraulic press. The dry celery powder was moistened with an equal volume of double distilled water and before extraction was placed in an ice box at a temperature of 12° C. for 16 hours. Two pressings of the material were made, the extract obtained in the second being labelled "residue" in the following

Table 10—pH Values of Celery Materials and of Carrot

Material Expressed:—Juice From	pH.	Material Expressed:—Water Extract	pH.
Carrot leaves	6.36	Healthy powder	5.33
Celery leaves	6.42	Blighted powder	6.08
Carrot residue	5.68	Blighted powder residue	5.00
Celery residue	5.66		

The hydrion concentration of this material was considerably higher than that at which the fungus made its best growth in culture. It is believed that the figures for "residue" more nearly represent the correct values for the true sap of the plant leaves, the liquid from the first being diluted by the wash water.

Oxygen Relations

To study the relation of the amount of air to the quantity of growth on corn meal agar slants, test tubes of various lengths and the same diameter were used. The tubes were closed with rubber stoppers and sealed with paraffin. Comparing the longest and shortest tubes a small difference in the amount of growth was noted. There was, however, no appreciable difference between the other tubes and checks all compared.

Plantings were then made on various media and covered with paraffin

oil. The fungus was apparently unchecked, making a white, cottony growth, most abundant at the plane of contact of the medium with the oil. In this plane the most mycelium was found on the glass of the enclosing test tube.

Much further investigation was evidently necessary. Making use of the H-tube method* the oxygen of the air above corn meal agar slants and carrot plugs was absorbed by means of alkaline pyrogallol, the tubes being quickly and carefully closed by rubber stoppers and paraffin. On the corn meal agar and all but one of the carrot plugs, absolutely no growth took place. One carrot culture showed a very slight growth at first but this was soon checked. In the control tubes, which were treated the same as the others except that the chemicals were not permitted to come in contact with each other, the cultures made an abundant growth completely covering the medium. The tubes were observed at intervals for several months. The stoppers were then removed and the mycelium plantings broken into pieces and placed upon freshly prepared corn meal agar slants. These were observed for two weeks, no growth taking place. The mycelium was evidently lifeless.

It is known that pyrogallic acid under the conditions of this experiment gives off a trace of carbon monoxide gas, which in sufficient quantity is detrimental and in larger proportions even lethal to many forms of life. To obviate this possible objection, test tube cultures were placed in atmospheres made of mixtures of carbon monoxide, carbon dioxide and air as shown in the tabulation. These atmospheres were obtained by displacing water in liter bottles with CO and CO_2 gases prepared by heating together oxalic and sulphuric acids. Where the CO_2 was not wanted, the mixture of the two gasses was bubbled through strong sodium hydroxide solution. Three corn meal agar slant cultures were first placed in the inverted bottle, the bottoms of the tests tubes resting on the flange of the bottle and the cotton plugs pressing tightly against the bottom. The mouth of the bottle was then put into a battery jar of water and the water made to rise to the mouths of the test tubes by removal of the air. This was accomplished by sucking out the air with a U-shaped glass tube, one arm of which extended upward to the bottom of the inverted bottle. In this way practically all the air, except that remaining in the test tubes, was removed and the water ready to be displaced by the gas desired. The desired atmospheres obtained, the bottles were closed under water by means of rubber stoppers. The tubes of each bottle were then shaken off the flange onto the bottom of the stopper, thus permitting free diffusion of the air of the tubes with the surrounding gases.

All the plantings grew except those in an atmosphere of half CO and half CO_2, (minus, of course, the 30-40 cc. of air contributed by the volume of each of the test tubes). The purpose of the experiment was thus accomplished. Carbon monoxide in relatively high concentrations is not inhibitive to the growth of the fungus.

*Giltner, W. A laboratory manual in general microbiology. 1916.

Table 11—Relation of Cercospora apii to Air, Carbon Dioxide and Carbon Monoxide

Atmosphere	6 Days' Growth Tubes			Growth after removal Tubes		
	1	2	3	1	2	3
½ CO–½CO_2	—	—	—	—	+	
⅓ CO–⅓CO_2–⅓ Air	Diam. .8 cm.	.8 cm.	.8 cm.	+ Pink color develops	+	+
½ CO–½ Air	+	+	+	+ Pink color develops	+	+
⅓ CO–⅔ Air	Diam. 2.0 cm. Pink	+ Pink	+ Pink	..... Pink		

It was noted in the above that a pink coloration appeared in the agar immediately before the fungus growth. This color extended into the agar about a centimeter. It gradually disappeared as the fungus made further growth although some remained as long as the cultures were kept in atmospheres containing CO. Microscopic examination of sections of the pink agar did not reveal the presence of any fungous hyphae. This pigment had on several previous occasions been noted under young cultures which to all knowledge had not been exposed to any gas. It was thought, however, that possibly some illuminating gas may have entered the tube during flaming. Accordingly an experiment which had the dual purpose of revealing something of the relation of the fungus to oxygen and oxygen diluting gases, and of the production, if possible, of the pink coloration by means of illuminating gas was carried out. The method used was exactly as described under the CO experiment. The gases used and their proportions were as indicated in the following table. The work was begun March 6, 1921, and the results recorded on the three dates shown.

The presence of the pink in the culture agar remained as long as the tubes were kept in the illuminating gas. The slight pink which was seen at first in other atmospheres disappeared while the tubes still remained in those gases. The fungus may have simply overshadowed it, used it in its metabolism, or excreted something which changed it into a leuco-form. Judging from this and the CO experiment it seems there is a possibility that the CO gas is responsible for the production of the color by the fungus. The CO content of the illumination gas used was 4.4 per cent. The poison gas, even a short exposure to it, probably so influences the organism that it excretes the something which is either colored or which reacts with the constituents of the nutrient producing the pigment. Time did not permit of more investigation of this interesting phenomenon.

Another experiment was performed to learn more concerning the relation of the fungus to atmospheric oxygen. Four corn meal agar slant cultures were placed into each of several chaucel flasks and the air exhausted by means of a May-Nelson vacuum pump. This quickly lowered the pressure to 3 mm. mercury, at which point it was held for 10 minutes. The glass cock was then closed and the pump stopped. Two cc. of water were placed in the bottom of each flask before the air

evacuation to prevent excessive drying of the agar. This gives practically an atmosphere of water vapor. The plantings were examined from time to time and no growth was observed. The checks showed normal growth, being 1 cm. in diameter 4 days after the planting and two weeks later completely covering the agar surface. Ten days after the beginning of the experiment, one of the flasks was tested with a mercury manometer and was found to have maintained its vacuum, the reading being 20 mm. or about the vapor pressure of water at the existing temperature. Air was admitted to this flask and its plantings examined 3 days later. A good growth was evident in all three tubes. For ten days the fungus had either been able to remain alive on the very small quantity of oxygen not removed from the agar and surroundings, or it is able to respire anaerobically for some time, or it can remain relatively dormant for this period. The air was again exhausted from the flask and growth stopped. It was reopened two weeks after the second evacuation. At the same time another flask, whose vacuum had been maintained continuously for 26 days, was tested and opened. Growth was not resumed in either flask, showing that two weeks' exposure to these conditions is fatal. The last flask was tested after a month's time and although it had leaked until the pressure was only 20 mm. lower than atmospheric pressure, the fungus in none of the tubes had grown. How long it had maintained a good vacuum it is impossible to say, but it probably leaked very gradually. Further experiments on the effects of air exhaustion and reduced pressure, more exact and conclusive, were not carried out because of lack of time.

Table 12—Growth in Various Gases: Comparison in Terms of Growth in Control Tubes

Atmosphere	GROWTH		
	3–9–21	3–13–21	3–18–21
All CO_2*	Slight. No pink color	Growth very slight. Concentric darkened rings	As 3–13–21. Shallow
¾ CO_2–¼ Air	¼ of check. No pink	As 3–9–21	do.
½ CO_2–½ Air	do.	do.	do.
¼ CO_2–¾ Air	½ check. No pink	do.	do.
All N_2*	Good growth. No pink	Thin spreading growth. No pink	½ check. Many dark brown sclerotium-like mats on surface
¾ N_2–¼ O_2	As check. No pink	Check	Check exactly
½ N_2–½O_2	do.	Check	⅔ check. No pink
¼ N_2–¾ O_2	Good growth. Slightly pink	Check	Check size. 1 cm. in med. Intense brown color at inner surface of growth
All O_2*	Good. No pink	As check, but slightly pink	¾ check. As above. No pink
All gas* (Lab. Illum.)	No growth. No pink	No growth	¼ check. Very pink
¾ Gas–¼ Air	⅔ check. Slightly pink	¾ check. Pink	¾ check. Pink
½ Gas–½ Air	½ check. Very pink	½ check. Pink	¾ check. Pink
¼ Gas–¾ Air	¾ check. Very pink	As check. Pink	As check. Pink
Check—All Air	.7 cm.	1.5 cm.	⅞ surface covered

All cultures had a few atypical spores.

*Except the air contained in the test tubes.

These experiments* demonstrate that the fungus, *Cercospora apii,* while able to maintain apparently normal growth in small quantities of free oxygen, is killed by the almost complete absence of this gas. Tolerance and reaction to the poisonous carbon monoxide gas was also noted.

Resistance to Desiccation

To determine the effect of drying on the life of the fungus a spore-mycelium suspension was made as described under the Thermal Death-point experiment. Using aseptic precautions, small, loose wisps of absorbent cotton were dipped into the suspension and then placed in sterile petri dishes. Several one-inch cover slips were covered with the suspension and placed in another petri dish. Both petri dishes were placed in a desiccator where they were allowed to remain until just the last trace of visible moisture vanished. The dishes were then removed and each, for protection against contamination, was placed in a cover of a deep culture dish which had been lined with cotton. The covers of the petri dishes were propped up on one side by means of sterile pieces of wire. Desiccation was allowed to proceed at room temperature. At the time intervals shown in the following table bits of the glass circles and cotton wisps were planted on corn meal agar slants.

Table 13—Relation of Cercospora apii to Desiccation

Glass Surface	Time of Drying—Days						
	4	8	16	22	32	38	68
Set No. 1	+	+	+	+	+	+	+
Set No. 2	+	+	+	+	+	+	+
Cotton Wisps	8	28	30	62			
Set No. 1	+	+	+	+			
Set No. 2	+	+	+	+			

+ indicates growth.

It is worthy of note that successful plantings were made from cultures which had been permitted to dry for fourteen months. The above results show simply the comparative tolerance of the fungus to desiccation when less protected.

To test the effect of drying on the conidia several blighted leaves were put in a desiccator for 36 hours and then removed to a petri dish where they were permitted to dry at room temperature. One edge of the petri dish cover was raised. At intervals of time shown below, spores were removed with forceps and placed in tap water on cover slips and germination observed as in the Thermal Death Point experiment. On the 15th day spores and conidiophores were mounted also from garden material that had been pressed the previous fall, placed in a pasteboard box and set in the specimen room. Both conidia and conidiophores germinated very readily. The material in the petri dish was consequently abandoned. Fruiting bodies were then taken from old herbarium material, dating from 1889 to 1912, and germination attempted.

*The writer is indebted to Dr. R. C. Huston of the Department of Chemistry for facilities for carrying on this experiment.

Table 14—Relation of Cercospora apii to Drying on the Celery Leaf

Mount	Time of Drying—Days								Mounts of Material 1889–1912
	1.5	3	4	6	7	9	13	170	
No. 1 Germination	+	+	+	+	+	+	+	+	—
No. 2 Germination	+	+	+	+	+	+	+	+	—

These results indicate great tolerance of the conidia to desiccation and the practical application of this to dissemination and infection is obvious. There were no indications whatsoever of living fungus in the mounts from the old herbarium specimens and, although allowed to remain several days in the moist chambers, no conidiophore or spore produced a germ tube.

DISSEMINATION

That air currents are to be considered as the main agents in the dissemination of Cercosporae and other fungi of this type is indicated in various writings (7, 15, 30). With a view toward determining the presence or absence of forcible explusion of Cercospora spores from conidiophores the following experiment was made. Using paraffin, corks were fastened to the lower surface of the tops of deep culture dishes. To the corks, about 5 mm. from the inner glass surface, were pinned small pieces of diseased leaves bearing an abundance of conidiophores and spores. Melted corn meal agar was then poured into the bottoms of the dishes and the tops replaced. Examined with the microscope the dry fruiting structures present the appearance of irregularly twisted, collapsed rubber tubes. Absorbing the moisture given off by the agar they rapidly become turgid and appear as when mounted in water. On being removed to the dry air again they very readily resume their original shrivelled appearance. Two microscopes were used in the observations. Examinations were made at varying intervals of time for a period of 36 hours. Spores were not observed to leave the conidiophores. The dishes were then set aside and examined a week later to see if any Cercospora colonies formed on the agar below. One colony had formed immediately below the leaf in one dish. The others, however, showed no growth. Apparently this fungus is provided with no means for the forcible expulsion of its conidia.

Wind distribution was demonstrated as follows: Pieces of blighted leaves were fastened to the inner top surface of a deep culture dish as described above. The upper surface of the glass immediately over the conidia was cooled by ether evaporation. Air was then blown onto the leaf by using a bent glass tube which passed down around the bottom of the microscope stage and up into the dish where it ended in a capillary opening. By using low power of the microscope and getting the lower surface of the glass in focus, spores were observed to appear

and stick in the condensation moisture which collected on the cool glass.

It should here be repeated that fungous conidia which had over-wintered in the garden were found incapable of germination the following spring. They are probably very rarely able to withstand the vicissitudes of winter conditions, being designed for distribution during the season in which they are produced.

VARIETAL RESISTANCE

The literature shows evidence of some little observation on the resistance or susceptibility to the fungus of a few celery varieties. Rolfs (1896) wrote that "self blanching varieties suffer worse than other kinds" (58). Davis (1893) recorded that "White Plume and Golden Self Blanching suffered more than other varieties. Henderson's New Rose has been entirely free from blight" (14). Taubenhaus (1918) stated that "Boston Market and Gold Heart should be avoided because of their susceptibility to the disease. The White Plume seems to be resistant" (71). Jones (1910) reported a Milwaukee celeriac grower as losing 90 per cent of his crop in 1909.*

Although observations, both in the field and the greenhouse, were numerous and records were made, they failed to reveal any marked resistance of any variety to the disease. Twelve different varieties of celery were used in these tests and there could be noted in the susceptibility to early blight no difference between celery of any one or several varieties.

CONTROL

Being a leaf spot disease it would seem that early blight would be amenable to control by a spraying of the plant foliage with some suitable fungicide. The published results of early trials are somewhat confusing. Halsted (1891) obtained good results with ammoniacal copper carbonate (33). Scribner (1886) found lime and sulfur of no value. He stated that "liver of sulfur may arrest its progress," but that he hesitated to recommend copper salts for "hygienic reasons" (63). Sturgis found sulfur dust superior to liver of sulfur solutions and the copper salt sprays (67-70). Atkinson (1893) recommended the use of ammoniacal copper carbonate. McCarthy (1895) in one of his six rules for combatting the blight wrote that "liver of surfur or sulfur flour may be applied in the seed bed after transplanting." Rolfs (1898) stated that the plants being "drenched twice a week with liver of sulfur solution gave complete control," as did the copper fungicides, which had the disadvantage of coloring the foliage. Hume (1900) preferred bordeaux to potassium sulfide and ammoniacal copper carbonate solution. Galloway (1893) wrote Davis of the Michigan station that celery on upland soil blighted badly in spite of the fact that the leaves were kept covered with bordeaux, ammoniacal copper carbonate and other fungicides, but that celery so treated was less attacked than the checks (14).

*From Plant Disease Survey Records.

Lockhead (1900) recommended spraying at two week intervals with ammoniacal copper carbonate.

Later investigators, as indicated in the pathologists' report, have come to the opinion that bordeaux is the most convenient and effective fungicide to be used in combating this disease. The reports are varied, "Bordeaux unsatisfactory," "Bordeaux and ammoniacal copper carbonate not very effective," "Bordeaux gives partial control," "Bordeaux good," and "Bordeaux gives excellent results," being some of the statements. The fact that California and Florida celery received here generally show early blight spots in spite of the leaves being coated by bordeaux, indicates that the spray as applied does not give complete control.

Spray experiments were run in the greenhouse with the object of determining the relative value of several fungicides in preventing early blight spotting. Preliminary to these experiments spores were placed in certain chemical solutions and observed for germination. Copper filings placed in distilled water, 4-4-50 bordeaux mixture, ammoniacal copper carbonate (5 oz. $CuCO_3$ and 3 pints 26° Baume ammonia to 50 gallons of water), mercury cyanide and bichloride (1-1000), and copper sulphate 1-128,000 inhibited the production of germ tubes. Lime sulfur (34° Baume made 1-45) and liver of sulfur (4 oz. to 10 gal. water) did not permit germination while the spores were in contact with the fresh solution, but on being allowed to dry and then wet again did not prevent some conidia from germinating. This may partially explain the spraying results obtained with potassium sulfide solution. Sulfur dust did not appear to inhibit germination. It is difficult to understand the good results obtained by Sturgis (70) with sulfur flour.

Lime-sulfur (1-40 and 1-45) was found injurious to the celery leaves, causing severe burning. It has, moreover, only a temporary effect in inhibiting conidial growth.

Of the several small tests of various spray mixtures run in the greenhouse the following is typical. Sets of six plants each were thoroughly sprayed, top and bottom of the foliage, with the respective fungicides as shown in the table, allowed to dry, and placed in the rain chamber. They were then sprayed with a spore-mycelium suspension made from 8 corn meal agar slant cultures and moistened from time to time with the mist. Seventeen days later the results expressed in the number of Cercospora spots per plant, were recorded. The identification of the lesions was confirmed by microscopic examination.

Table 15—Efficiencies of Various Spray Materials in Early Blight Control

Spray Material	Number of spots per plant					
Bordeaux	3	2	1	2	12	10
Ammon. $CuCO_3$	4	18	4	27	10	30
K_2S	33	26	50	20	16	..
Lime Sulfur*	10	12	18	8	3	25
Checks	4 plants used showed 40–60 spots each.					

*Severe burning with this fungicide on all plants.

The relative value of the four fungicides, in this small experiment at least, is at once seen. The bordeaux mixture has a decided advantage over the other solutions. Because of the growth of the celery, exposing new areas untouched by spray, one should not expect absolute freedom from the disease. However, it is evident that benefits are derived from a thorough application of an efficient fungicide. In regions where this disease is known to be bad and during the seasons when the climatic conditions are described as unfavorable for celery, spraying with 4-4-50 bordeaux mixture is advisable. The applications should begin in the seed bed, should be very thorough, covering both leaf surfaces, and should be given at intervals of a week or ten days. This carries double protection as the Septoria blight is also controlled by such applications.

The observation of many is that early blight is very uncommon on celery grown in rich soils that have plenty of water. Celery grown on the cool, moist Kalamazoo muck, has rarely been badly attacked by this fungus. Galloway's experiments are very indicative (28, 29, 14). The celery grown on an experimental plot at Mason was unusually large and thrifty. This celery had been watered by flooding. It showed no evidence of early blight. That raised at East Lansing and watered by the overhead system, produced large edible stalks in spite of the leaves being badly spotted, by the disease. It appears that, given an adaptable, fertile soil, water is a very important factor in controlling early blight. Where it is possible to irrigate thoroughly or flood the land during the hot, dry parts of summer, spraying may be unnecessary or not of sufficient value to be economically important. However, as the season progresses toward the time of harvest, spraying for the purpose of producing unspotted, more attractive foliage, and what is still more important, as an insurance against the destructive late blight, is advisable.

After harvest, to remove the big source of infection to the following crop, the field should be cleaned of all refuse. Infected material would best be burned.

SUMMARY

Celery early blight is a disease of celery and celeriac known practically wherever these plants are grown, in Europe and America. It is caused by a specific fungus, *Cercospora apii,* which was first described by Fresenius in 1863. The disease appears as suborbicular spots on the leaves and also, but not commonly, on the stems. In times of high humidity the lesions are covered with the fruiting bodies which give a velvety appearance.

Given high temperatures supplemented by heavy evening dews, a dry soil and consequently plants of low vitality, the disease may become of great economic importance.

The fungus enters its host through open stomata by means of conidial germ tubes. It grows intercellularly, absorbing nutriment from the surrounding tissue by means of haustoria. As the food components of a lesion become exhausted, the fungus thrusts through the stomata, in fascicles, long fruiting hyphae, each of which bears a long obclavate spore at its end. When mature these spores are easily detached and distributed by air currents. Wind distribution was demonstrated.

The organism grew well on all media employed, including sterile muck and refuse. Spores were capable of germination in the range pH 4.6 to pH 8.0 in some buffer solutions.

A study of the temperature relations of the fungus showed that the parasite made its largest vegetative growth between 25° C and 30° C, that an exposure of 48° C for 10 minutes was fatal to the conidia and 51° C. to the conidiophores and mycelium. Spores germinated best at 27-29° C. There was no germination after 12 hours exposure at 42° C.

Fourteen months desiccation was not lethal to mycelium, and spores were found capable of germination after six months drying at room temperature.

Though requiring some free oxygen for life, the fungus was very tolerant in this regard.

A study of infection on 12 different varieties of celery showed no varietal resistance to early blight.

Control is a matter of keeping the plants vigorously growing, that is, on soil of high fertility and good drainage and having an abundance of moisture. This should be supplemented by spraying with bordeaux mixture as indicated.

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Plate I. Early Blight of Celery. (Photo by Dr. G. H. Coons)

Plate IIa. Plants diseased as a resnlt of shaking over-wintered diseased material over them.
(Photo by Mr. R. Nelson)

b. Cultures on sterilized muck and refuse.
(Photo by Mr. R. Nelson)

Plate III. Naturally infected plant showing broken, diseased, outer stems.

(Photo by Dr. G. H. Coons)

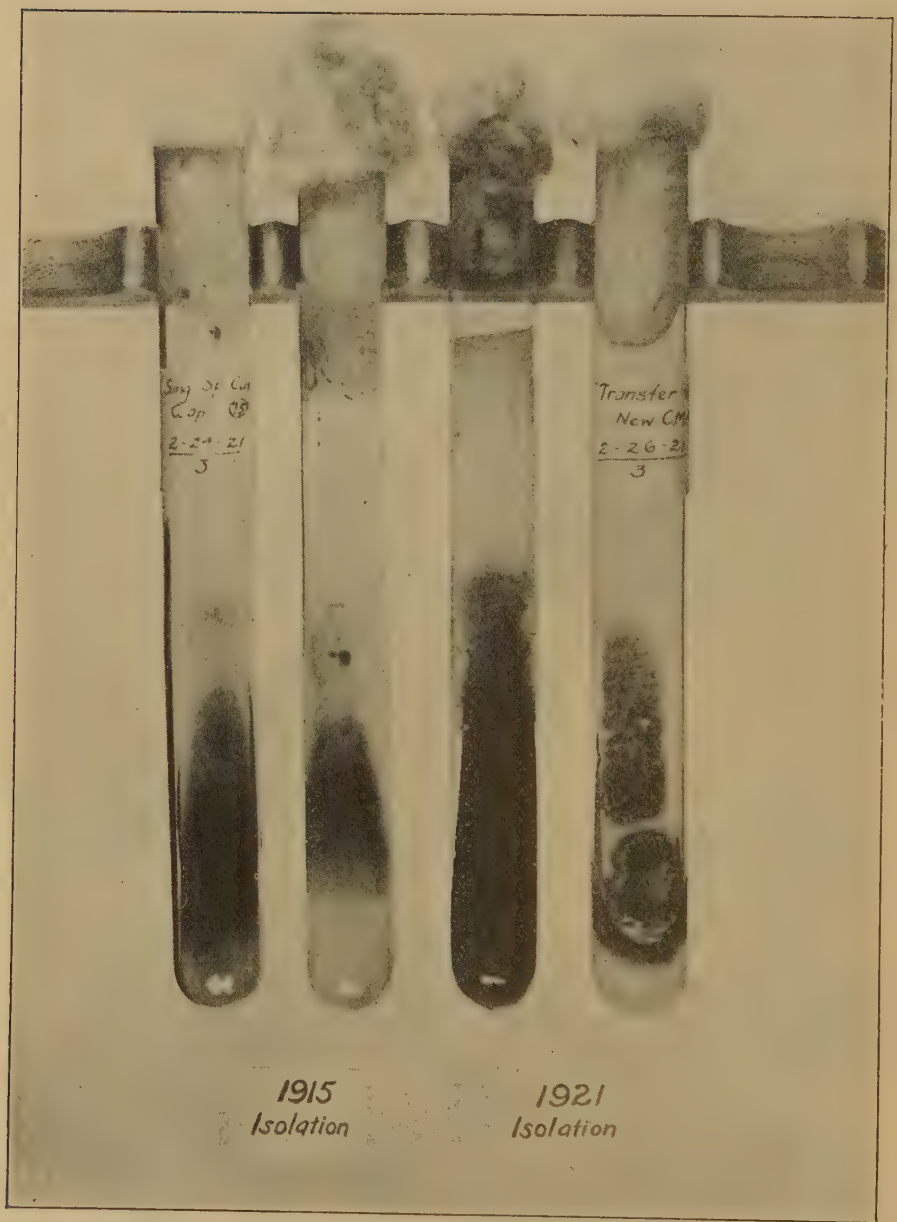

Plate IV. Corn meal agar cultures of the Celery Early Blight organism, *Cercospora apii* Fres.

(Photo by Mr. J. E. Kotila)

Plate V. *Cercospora apii.* Camera lucida drawings of—

Fig. 1.—Germ tubes from mycelium entering stoma of celery leaf.
Fig. 2.—Conidiophores of the fungus protruding through a stoma (young stage).
Fig. 3.—Mycelium from corn meal broth culture having H-ion concentration pH 8
Fig. 4.—Mycelium from diseased leaf showing knob-like haustoria.
Fig. 5.—Gnarly, tortuous mycelium from culture on Melilotus stems.
Fig. 6.—Sclerotium-like bunches of mycelium formed in corn meal broth pH .5 and pH 8.

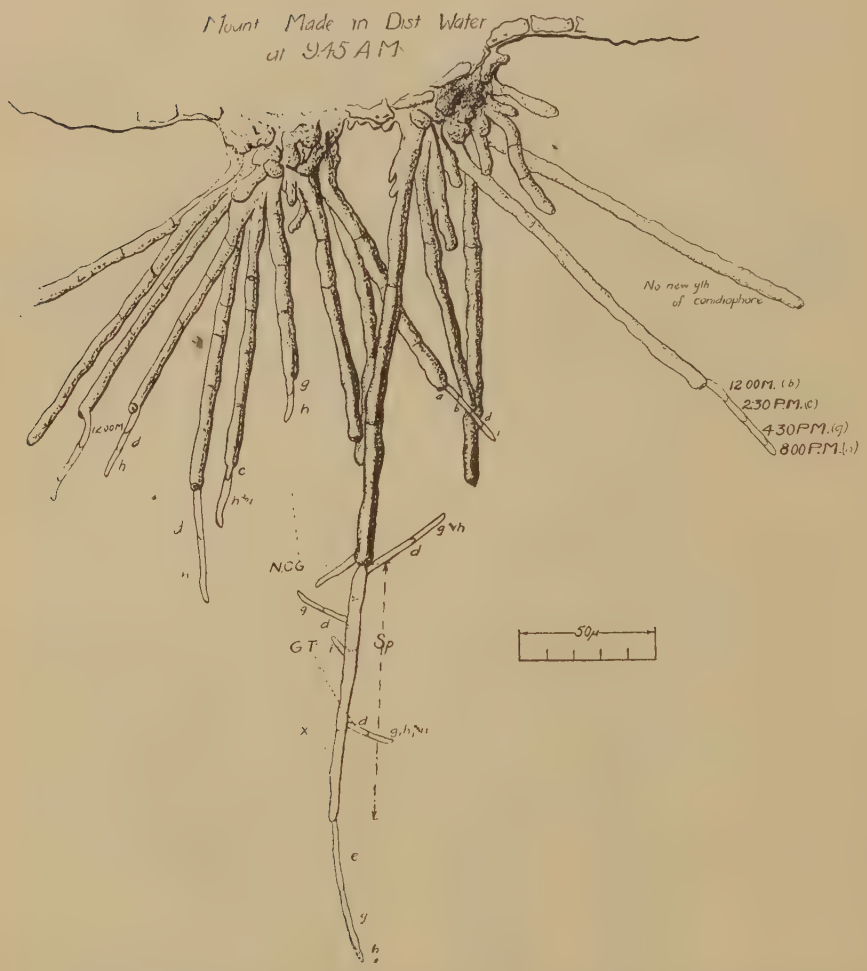

Plate VI. Growth rate of *Cercospora apii*. Camera lucida tracings of conidiophore fascicles (advanced stage) showing spore (Sp.) germinating *in situ*, and new conidiophore growths from each of which another spore would have been abscissed.

N. C. G.=New conidiophore growth.

G. T.=Germ tube.

Small letters refer to time showing rate of growth. The mount was made at 9:45 A. M.

a—11:00 A. M.
b—12:00 M.
c—1:00 P. M.
d—2:00 P. M.
e—2:30 P. M.
f—4:15 P. M.
g—4:30 P. M.
h—8:00 P. M.
i—5:00 A. M. (next day).

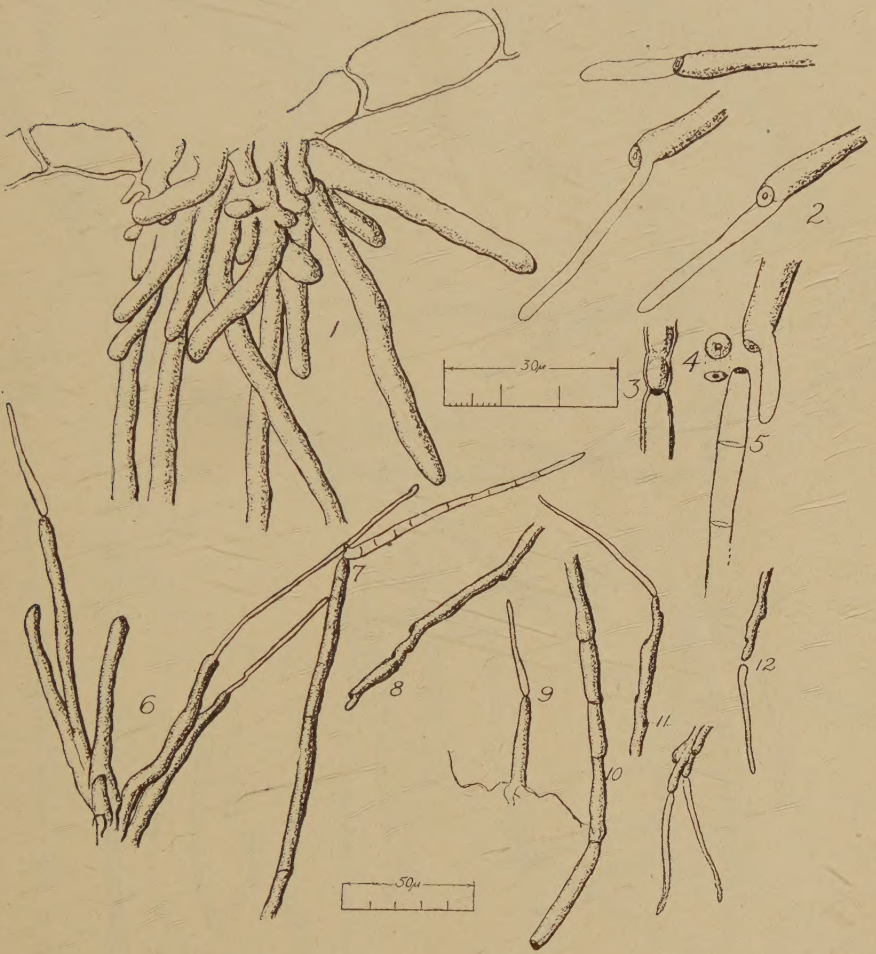

Plate VII. *Cercospora apii* conidiophores. Camera lucida drawings of—
Fig. 1.—Base of conidiophore fascicle (Oil immersion).
Fig. 2.—Tracings of conidiophore tips showing characteristic scars and new growth (oil immersion).
Figs. 3, 4, 5.—Showing attachment of conidium to conidiophore (Oil immersion).
Figs. 6–12.—Tracings of permanent mounts (paraffin method), showing conidiophores, spores, scars and geniculations.

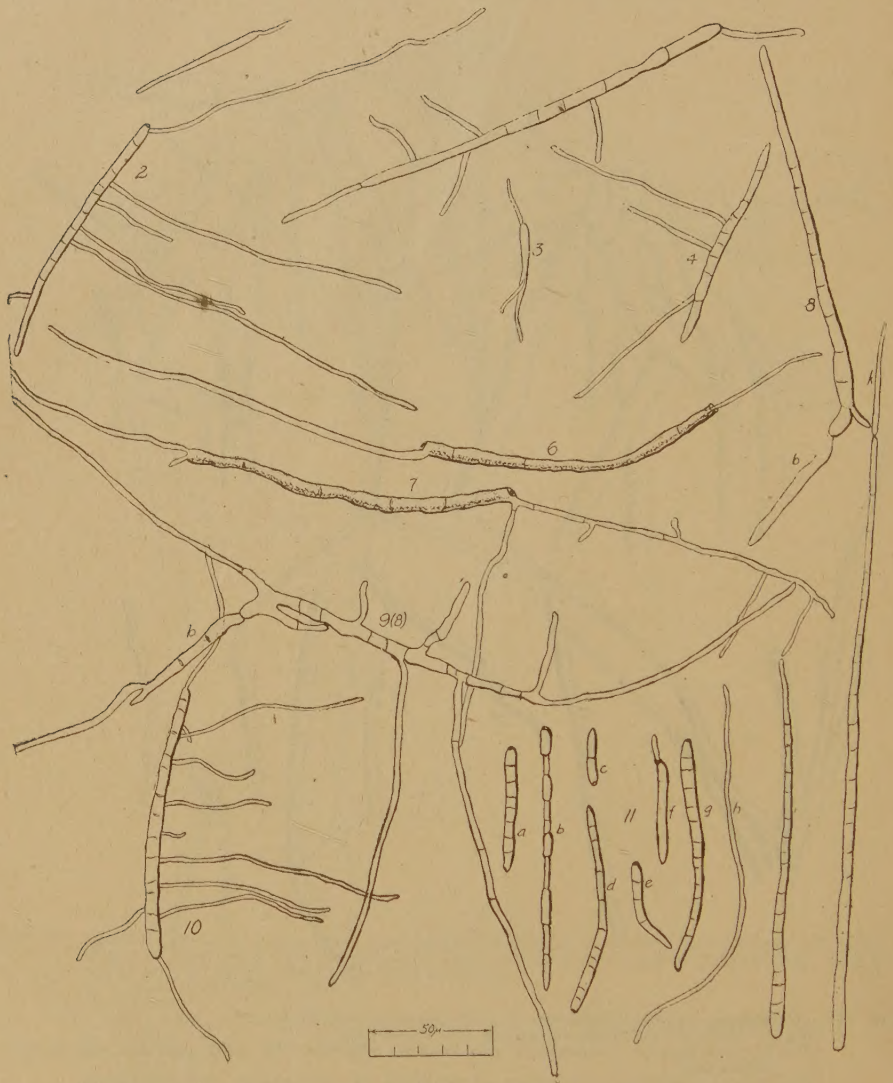

Plate VIII. Cercospora apii. Camera lucida drawings (high power) of—
Figs. 1–5, 8, 9, 10.—Germinating conidia.
Figs. 6, 7.—Germinating conodiphores.
Fig. 11.—(a to g, i) Forms of conidia.
Fig. 11h.—An atypical spore from pure culture.
Fig. 11 j, k.—Two attached conidia showing tendency toward chain formation.

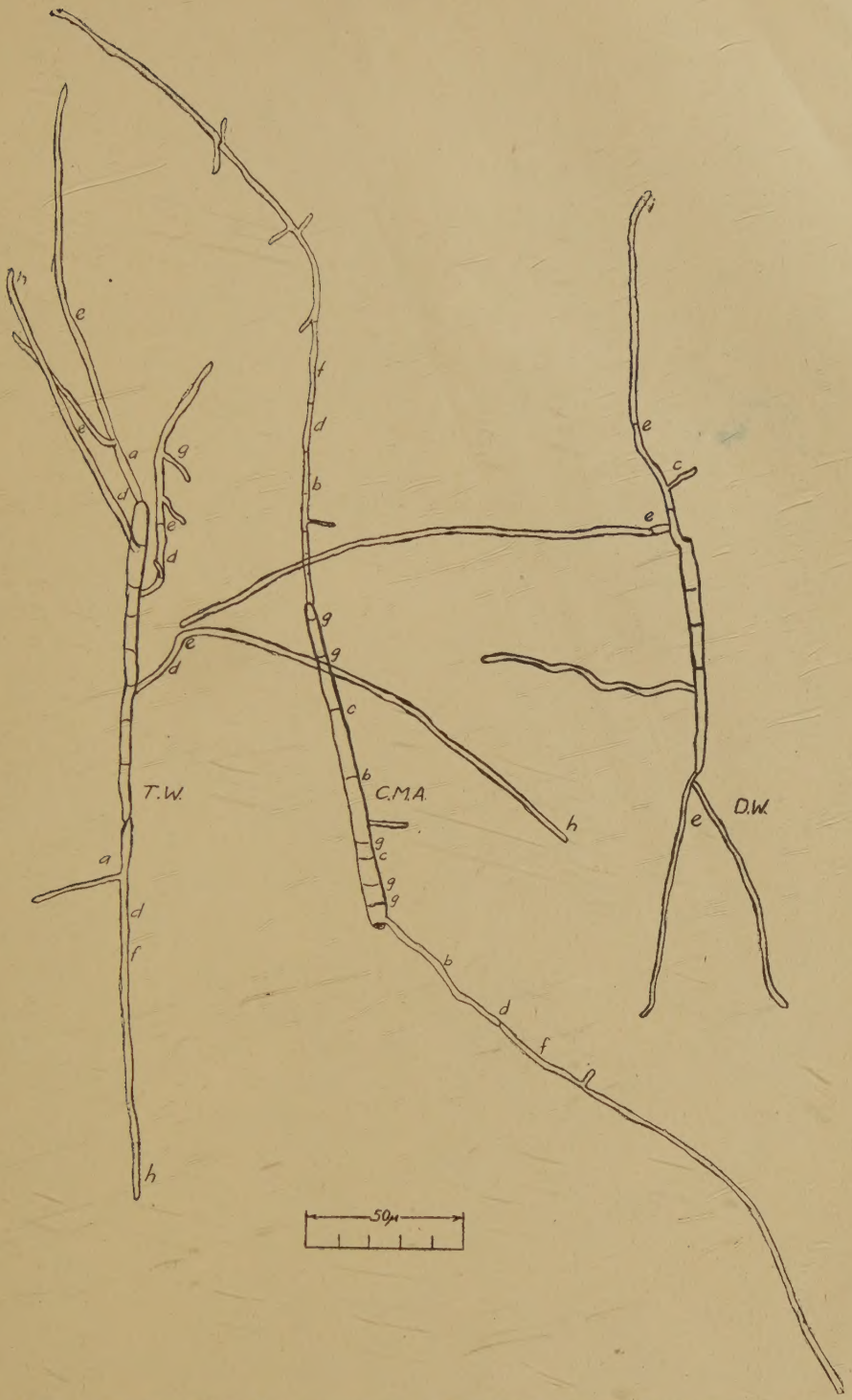

Plate IX. Spore germination of *Cercospora apii*. Camera lucida drawings (high power) of germinating spores, showing relative rate of growth of germ tubes in tap water, distilled water, and corn meal agar. The experiment was begun at 9:00 P. M., and the temperature was 28–30° C. The small letters refer to time.

a—10:30 P. M.	d—5:00 A. M.	g—9:00 A. M.
b—12:00 P. M.	e—7:00 A. M.	h—9:45 A. M.
c—2:00 A. M.	f—7:30 A. M.	i—11:00 A. M.